Hit List

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Search Results - Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: US 5506121 A

L11: Entry 1 of 1 File: USPT Apr 9, 1996

US-PAT-NO: 5506121
DOCUMENT-IDENTIFIER: US 5506121 A

TITLE: Fusion peptides with binding activity for streptavidin

DATE-ISSUED: April 9, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Skerra; Arne	Wiesbaden			DE
Schmidt; Thomas	Luxembourg			DE

US-CL-CURRENT: 435/69.7; 435/252.3, 435/320.1, 530/350, 530/412, 536/23.4

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Terms	Documents
5506121.pn.	1

Display Format: CIT Change Format

Previous Page Next Page Go to Doc#

Invention Paragraph:
[0044] Within the scope of the invention it was found that the peptide sequences of the invention have high binding affinity for streptavidin or nuclear streptavidin (a proteolytic cleavage product of streptavidin) (Bayer, E. A., et al., Biochem. J. 259 (1989), 369-376) and for streptavidin muteins, which affinity is in particular higher than the binding affinities of the individual binding modules; at the same time the said peptides can readily be eluted under competitive conditions.

Summary of Invention Paragraph:
[0045] The individual binding modules of the peptide of the invention preferably have a binding affinity K.sub.d for the particular streptavidin receptor of not more than 10.sup.-2, more preferably not more than 10.sup.-3, even more preferably not more than 10.sup.-5 and at least 10.sup.-13, more preferably at least 10.sup.-10, even more preferably at least 10.sup.-8 and most preferably at least 10.sup.-6 M. Elution under competitive conditions is then preferably carried out using a competitor which has a higher affinity for the particular streptavidin receptor, preferably an affinity which is at least one order of magnitude, more preferably at least two orders of magnitude and most preferably at least three orders of magnitude greater. The binding affinity of streptavidin/biotin, for example, is 4.times.10.sup.-14 M. Owing to the sequential arrangement of the inventive ditags and of the avidity effect connected therewith the use of 2 binding modules which in each case have a binding affinity of 10.sup.-6 M or higher, in particular 10.sup.-5 M or higher, is in particular possible, and despite the low binding affinities of the individual binding modules a strong binding to the streptavidin receptor is obtained under non-competitive conditions.

Summary of Invention Paragraph:
[0046] Particular preference is given to those streptavidin muteins which are described in U.S. Pat. No. 6,103,493 and also in DE 196 41 876.3. These streptavidin muteins have at least one mutation within the region of amino acid positions 44 to 53, based on the amino acid sequence of wild-type streptavidin. Preference is given to muteins of a minimal streptavidin, which start N-terminally in the region of amino acids 10 to 16 of wild-type streptavidin and end C-terminally in the region of amino acids 133 to 142 of wild-type streptavidin. Examples of such streptavidin muteins have a hydrophobic aliphatic amino acid instead of Glu at position 44, any amino acid at position 45, a hydrophobic aliphatic amino acid at position 46 or/and a basic amino acid instead of Val at position 47. Particular preference is given to streptavidin muteins having the sequence Ile-Gly-Ala-Arg or Val-Thr-Ala-Arg at amino acid positions 44 to 47.

Summary of Invention Paragraph:
[0047] The isolated peptide of the invention is preferably used as label (tag) or affinity tag. The invention therefore further relates to a fusion protein comprising an inventive peptide as described above which has at least two individual modules binding to streptavidin or streptavidin muteins linked to a protein. If the peptide sequence of the invention is present in a fusion protein, this fusion protein, too, has a high affinity for streptavidin and can at the same time be readily eluted under competitive conditions.

Summary of Invention Paragraph:
[0048] Besides eluting under competitive conditions, i.e. in the presence of another streptavidin ligand, it is also possible to break the receptor:affinity tag interaction by changing the pH (pH shift), which makes simple elution possible. Under acidic conditions at least one histidine residue of the peptide of the invention is protonated resulting in the breaking up of the receptor:affinity tag interaction.

Summary of Invention Paragraph:
[0051] In a further embodiment, the invention refers to a method for detection of a binding event between a protein and an analyte that is capable of binding to the protein by use of a biochip or biosensor. In this method a biochip/biosensor is used on which surface streptavidin or a streptavidin mutein is immobilized. The immobilization of streptavidin or the streptavidin mutein on the surface of the biosensor can occur by any suitable immobilization method and/or coupling chemistry, for example, using the protocol describes by Busch et al. (2000). For one embodiment of this method, the mutein known as Strep-Tactin.RTM. is preferably used.

Summary of Invention Paragraph:
[0052] This method of detection of a binding event between a protein and an analyte takes advantage of the strong binding affinity that the peptide of the invention has to streptavidin or a streptavidin mutein. The method comprises as step (a) contacting a first sample containing a protein which is linked (fused) to a peptide of the

present invention (i.e. a peptide that comprise the sequential arrangement of at least two different or identical streptavidin-binding or/and streptavidin mutein-binding modules) with the biosensor, thereby allowing the formation of a complex between said protein and streptavidin or a streptavidin mutein via the peptide of the invention. Step (b) is contacting the biosensor with a second sample which can contain an analyte which analyte is capable of binding to said protein fused to the peptide of the invention, thereby allowing the formation of a complex between said protein fused to the peptide of the invention and the analyte, and in step (c) binding of the analyte to the protein is detected by use of a signal caused by the formation of the complex between said protein fused to the peptide of the invention and the analyte.

Summary of Invention Paragraph:
[0056] In a further preferred embodiment of this detection method the signal caused by the formation of the complex between said protein and the analyte is a surface plasmon resonance signal. Accordingly, the biosensor is preferably a so-called Biacore.RTM.-chip. The signal can, however, be also generated by a fluorescence or chromogenic label which is conjugated to one of the two complex partners. An advantage of the use of ditag or multitag of the present invention in an detection based on biosensors is that the biosensor can easily be regenerated. For this purpose, the protein immobilized on the surface of the chip via the ditag or multitag disclosed here is simply removed by washing the chip surface with a competitive streptavidin or streptavidin mutein ligand such as diaminobiotin and/or desthiobiotin. One such regenerated chip can thus be loaded with many different

Brief Summary Text (10):
A disadvantage of this system has previously been its relatively low affinity. An affinity constant of 2.7.times.10.sup.4 M.sup.-1 has been determined by means of isothermal titration calorimetry for the complex between streptavidin and the peptide ligand referred to as strep-tag (Ala Trp Arg His Pro Gln Phe Gly Gly (SEQ ID NO: 1)). Although there were indications that the binding could be somewhat stronger for a fusion protein containing the peptide ligand, it is desirable to have a system with a fundamentally improved affinity.

Brief Summary Text (23):
For practical considerations it is desirable to have a further ligand which, due to a higher binding affinity or/and when present at higher concentrations, can detach the binding of the previously defined peptide ligands (according to DE-OS-4237113) from the streptavidin mutein according to the invention. In this manner it is possible to release bound peptide ligands or proteins to which a peptide ligand is fused under very mild elution conditions. Hence under this aspect the present invention concerns those streptavidin muteins according to the invention whose binding affinity for peptide ligands is such that they can be competitively eluted by other streptavidin ligands e.g. biotin, iminobiotin, lipoic acid, desthiobiotin, diaminobiotin, HABA (hydroxyazobenzene-benzoic acid) or/and dimethyl-HABA. The use of coloured substances such as HABA has the advantage that the elution can be checked visually.

Brief Summary Text (29):
A further aspect of the present invention concerns a cell which is transformed or transfected with such a vector which carries as an insert at least one copy of a nucleic acid sequence coding for a streptavidin mutein according to the invention. The selection of the cell is not particularly critical and in general it is possible to use any cells that are suitable for such purposes. Prokaryotic as well as eukaryotic cells and yeasts come into consideration. For practical reasons prokaryotic cells are generally preferred and in particular E. coli for the expression of an unglycosylated protein as in the present case.

Brief Summary Text (36):
The advantages over the conventional streptavidin/biotin system apply in particular to affinity chromatography and in purification, isolation or determination methods for recombinant proteins. Accordingly the invention also concerns the use of a streptavidin mutein according to the invention in a method for the isolation, purification or detection of a protein that is fused with a peptide sequence of the formula Trp-Xaa-His-Pro-Gln-Phe-Xaa-Xaa (SEQ ID NO: 16) in which X represents an arbitrary amino acid and Y and Z either both denote Gly or Y denotes Glu and Z denotes Arg or Lys wherein a liquid containing the protein to be isolated or purified is contacted with the optionally immobilized streptavidin mutein under suitable conditions in order to bind the peptide sequence to the streptavidin mutein, the resulting complex is separated from the liquid and the protein is released from the complex or detected. The peptide sequence is particularly preferably selected in the form of strep-tag or strep-tag II. The peptide sequence is preferably fused to the N- or/and C-terminus of the protein. The streptavidin mutein can be bound to a solid phase or can be capable of binding to it.

Brief Summary Text (37):
An advantage of utilizing the streptavidin mutein/peptide ligand system according to the invention in an isolation or purification method is that very mild conditions can be used to elute the fusion protein carrying the peptide ligand. Hence it is possible to incubate a solid phase coupled to the streptavidin mutein, such as for example an affinity chromatography column to which the fusion protein has been adsorbed, with an adequate concentration of a ligand selected from biotin and derivatives thereof in order to release the fusion protein from the complex. In this connection the use of desthiobiotin has proven to be particularly advantageous.

Brief Summary Text (38):
The streptavidin muteins according to the invention can be used in detection methods in an essentially similar manner to the corresponding methods that are known for conventional streptavidin. A further application is the qualitative or quantitative determination of a protein which is fused with a peptide sequence of the formula Trp-Xaa-His-Pro-Gln-Phe-Xaa-Xaa (SEQ ID NO: 16) in which X represents an arbitrary amino acid and Y and Z either both denote Gly or Y denotes Glu and Z denotes Arg or Lys, wherein the protein to be determined is contacted under suitable conditions with a labelled streptavidin mutein in order to bind the peptide sequence to the streptavidin mutein and the label is determined. Such a determination method can for example be carried out qualitatively to detect proteins in Western blots or quantitatively as an ELISA. Suitable labels are all known radioactive and non-radio-active labelling groups e.g. luminescent groups, enzymes, metals, metal complexes

etc. The streptavidin can be directly labelled e.g. by covalent coupling. However, indirect labels such as labelled anti-streptavidin antibodies or biotinylated enzymes etc. can also be used.

Brief Summary Text (39):
A further subject matter of the invention is the use of the streptavidin muteins according to the invention to immobilize a protein which is fused with a peptide sequence Trp-Xaa-His-Pro-Gln-Phe-Xaa-Xaa (SEQ ID NO: 16) in which X represents an arbitrary amino acid and Y and Z either both denote Gly or Y denotes Glu and Z denotes Arg or Lys. This immobilization is preferably carried out on solid phases coated with streptavidin muteins such as microtitre plates, microbeads made of organic or paramagnetic materials or sensor chips.